



Indian Journal of Experimental Biology
Vol. 58, April 2020, pp. 286-291



Assessment of genetic fidelity in microclones of curry leaf plants [*Murraya koenigii* (L.) Spreng.] using ISSR markers

Nisha Khatik^{1*} & Ramesh Joshi²

¹Department of Botany, Maharshi Dayanand Saraswati University, Ajmer-305001, Rajasthan, India

²Plant Biotechnology Laboratory, Department of Botany, S. P. C. Government College, Ajmer-305001, Rajasthan, India

Received 30 October 2018; revised 25 February 2020

Murraya koenigii (L.) Spreng., commonly called Indian curry leaf, is an aromatic shrub highly valued for its medicinal potential viz. anticonvulsant, antitumor, anti-inflammatory, antidiabetic, antiviral and diuretic. Its leaves, locally known as curry patta, are used widely for culinary purpose, particularly in India. However, *M. koenigii*, due to poor rate of fruit set and seeds with short viability period and poor rate of germination, are sparsely distributed in wild, and has attracted researchers for propagation through biotechnological approaches. In this context, maintaining genetic fidelity is a vital for assessing genetic uniformity in micropropagated plantlets as variations within the progeny can result in serious losses to the end users. Therefore, it is necessary to screen them for their genetic makeup whether they are true-to-type or not. Molecular techniques like Inter Simple Sequence Repeat (ISSR) that are not influenced by environmental factors are appropriate tools to analyse genetic fidelity of *in vitro* propagated plants as, and generate reliable and reproducible results. In this study, we tried to evaluate genetic fidelity of micro-clones of tissue culture raised *M. koenigii* using ISSR technique. Twenty five ISSR primers were used to amplify genomic DNA from *in vitro* raised field grown plants and mother plant. Out of 25 primers screened, a total 465 amplified products were obtained from 10 ISSR primers. Out of which, 55 were monomorphic across the mother plant and its micropropagated progenies of 10 ISSR primers 05 showed profiles identical to mother plant. Similarity matrix based on Jaccard's coefficient and pair-wise values between mother plant and tissue cultured plant ranged from 0.91 to 1.00, indicating a high degree of genetic fidelity.

Keywords: Culinary, Indian curry leaf, Indian spices, Molecular markers, Traditional medicine

Murraya koenigii (L.) Spreng. (Fam.: Rutaceae), commonly known as Indian curry leaf tree, is a native of the Indian subcontinent. It shares aromatic nature, more or less deciduous shrub or tree up to 3-5 meters in height and 15 to 40cm in diameter with short trunk, thin smooth grey or brown bark and dense shady crown, and are used in the traditional system of medicine in Eastern Asia^{1,2}. The *Murraya* species is the richest source of carbazole alkaloids, reported for their various pharmacological activities such as anticonvulsant, antitumor, anti-inflammatory, diuretic, antiviral and activities³. *Murraya* leaves are full of antioxidants, namely, tocopherol, β -carotene, and lutein, and possess antioxidative activities, providing protection against oxidative stress^{4,5}. Koenidine, a metabolically stable carbazole alkaloid isolated from the leaves of *M. Koenigii* demonstrated a considerable reduction in the postprandial blood glucose level and

improved insulin sensitivity in streptozotocin-induced diabetic rats⁶.

In order to cater the increasing demands of herbal drug market, conservation and commercial production of these plants have become necessary. Since, conventional methods of propagation are generally slow, labour intensive, requiring large number of propagules and limited success, modern methods of biotechnology have taken lead in propagation of plants^{7,8}. Consequently, micropropagation has now become an important thrust area for medicinal plant research⁹⁻¹². Clonal propagation and preservation of elite genotypes, selected by their superior characteristics, require high degree of genetic uniformity among the regenerated plants. In medicinal plants, it is all the more important to ensure that there is no variation in the amount and quality of active principles. Tissue culture is an efficient method of clonal propagation; however, the resulting regenerants often has a number of somaclonal variations¹³. Occurrence of somaclonal variation is a disadvantage

*Correspondence:

E-mail: drnishakhatik14@gmail.com;

drrameshjoshi10@gmail.com

for both *in vitro* cloning as well as germplasm preservation method, and therefore, the investigation of genetic variability/stability of *in vitro* plants is extremely important.

Among various pathways of micropropagation enhanced axillary, adventitious branching, somatic embryogenesis is generally considered to be immune to genetic changes that might occur during cell division or differentiation^{14,15}. There are several cases where genetic stability has been demonstrated in adventitious shoot bud, callus and cell suspension culture regenerants¹⁶⁻¹⁸. However, there are several examples where instability has been reported in plants regenerated from highly organized state of shoot apex and meristem cultures^{19,20}.

DNA based molecular markers are reliable source to access the genetic variability of *in vitro* regenerated plants. These are not influenced by environmental factors, and hence generate reliable and reproducible results. ISSR markers comprise a few highly informative multi-allelic loci. They provide highly discriminating information with good reproducibility, and are relatively abundant^{21,22}. Inter simple sequence repeat (ISSR) has been mostly favoured and successfully applied to detect genetic similarities or differences in tissue-cultured materials of various plants. Since ISSR markers amplify different regions of the genome, their use allows better analysis of genetic fidelity/stability of the plantlets²³⁻²⁶.

ISSR analysis was effective to eliminate the somaclonal variant in *in vitro* leaf-derived horseradish plants²⁷. A few informations are available on genetic relationships among wild and cultivated accession in *M. Koenigii*²⁸ and its relatives²⁹ but as such no reports are available on the molecular evaluation of genetic fidelity in micropropagated plants of *M. koenigii* using ISSR markers. Hence, in this study, we have made an attempt to evaluate genetic fidelity of micropropagated plants of *Murraya koenigii* produced axillary bud, inter-node explants from approximately 10-year old matured plant and leaf, cotyledons, cotyledonary node (embryonic axis), hypocotyls and root segments from *in vitro* raised seedlings³⁰ using ISSR markers.

Material and Methods

Genomic DNA extraction and PCR amplification

Regenerants developed from all the seven type of explants (may be mentioned again here for clarity)

were selected randomly for genetic fidelity assessment. Genomic DNA from leaves of regenerants and mother plant (P) was extracted using CTAB method³¹. The total genomic DNA was quantified spectrophotometrically (UV-Vis Spectrophotometer, Pharmaspec UV-1700, Shimadzu, Japan) and aliquots were diluted to the final concentration of 10-15 ng μL^{-1} . PCR reaction for ISSR was performed in a programmable thermal cycler (Master cycle egradient S, Eppendroff, Germany). A total of 25 ISSR primers of 800P and UBC series (Eurofins Genomics, India) were initially screened for their applicability in PCR amplification of total genomic DNA of *M. koenigii*. Out of 25 ISSR, only 10 produced clear and reproducible amplified products.

ISSR profiles were produced through PCR amplification using the protocol described by Verma & Rana²⁸. PCR amplification was carried out in 25 μL volume using 10 different decamer primers. The reaction buffer consisted of 2.5 μL of 10X PCR buffer, 2.0 μL MgCl_2 (2.5 mM), 0.50 μL dNTPs (10 mM each of dATP, dGTP, dTTP and dCTP) (Bangalore Genei, India), 2 μL primer, 0.2 μL DNA Taq polymerase (*In vitro*gen platinum), 5 μL DNA sample and 12.80 μL water. The primers showing polymorphic bands were then used for analysing the clonal fidelity of micro-propagated plants.

For all the samples, PCR programme involved an initial denaturation at 94°C for 04 min followed by 35 cycles of 1.0 min denaturation at 94°C, 1.0 min primer annealing at 52°C, 2 min primer extension at 72°C and final extension for 7 min at 72°C. The amplified PCR-ISSR products were electrophoresed in 1.5% agarose in 1X TBE (90 mM Tris-borate, 2 mM EDTA, pH 8.0) buffer. The gels were stained with ethidium bromide and documented using gel documentation system (Expert Vision, Mumbai, India).

Data analysis

For *in vitro* plantlet regeneration, all the experiments were set up in a Randomized Block Design (RBD). Each experiment was repeated thrice with minimum 10 replicates per treatment. The data were analyzed statistically using SPSS ver. 7.5 (SPSS Inc., Chicago, USA) and the results are express as means \pm SD³². For genetic fidelity analysis, the fingerprints were scored considering fragment size at a locus as bi-allelic (present = 1, absent = 0). Reactions with each primer were repeated at least thrice and only those fragments

that were well resolved and reproduced in each instance were scored and included in the analysis ignoring the bands intensity. ISSR profiles obtained for *in vitro* derived regenerants were compared with mother plant.

Results and Discussion

Genetic fidelity assessment using ISSR primers

In this study, ISSR marker were used to characterize and analyze the genetic fidelity between mother plant and micropropagated plants of *M. koenigii* were also analyzed using 25 ISSR primers of which 10 generated amplified products. Out of 10 ISSR markers tested 05 produced reproducible polymorphic banding patterns. In 800 P series of ISSR primers, 811 P and 826 P primers didn't show any polymorphism and they generated 40 and 60 loci with band range size between 250 and 850 bp, and 100 and 1500 bp, respectively. Primer 818 P and 815 P produced 63 and 60 bands with 12.5 and 22.22% polymorphism respectively (Fig. 1 A-B).

Total five ISSR primers of UBC series were tested out of which three didn't generate polymorphic bands. In UBC series of ISSR primers, the highest percentage of polymorphism 50% with a 24 amplified bands were generated by UBC 857 primer in which the band size was ranging between 400 and 1000bp (Table 1), which in followed by 815 P (22.22%) > 814 P (20%) > UBC 881 (14.29%) and lowest percentage of polymorphism was observed in 818 P (12.50%). Hence, the observations of present study on the basis of ISSR, fingerprinting patterns resulted that the micropropagated plant of *Murraya koenigii* showed genetic fidelity with their mother plant.

Similarity coefficient Analysis of ISSR Profiles

Jaccard's pair-wise similarity coefficient values among micropropagated and mother plants of *M. koenigii* were ranged from 0.91 to 1.00 (Table 2). In our study, 91% similarity coefficient was observed in maximum number of samples screened. The highest similarity (98%) was observed between P¹-P⁵, P¹-P⁶, P⁵-M and P⁶-M, while the lowest value 0.91 was obtained between P¹-P², P¹-P⁴ and P⁴-M.

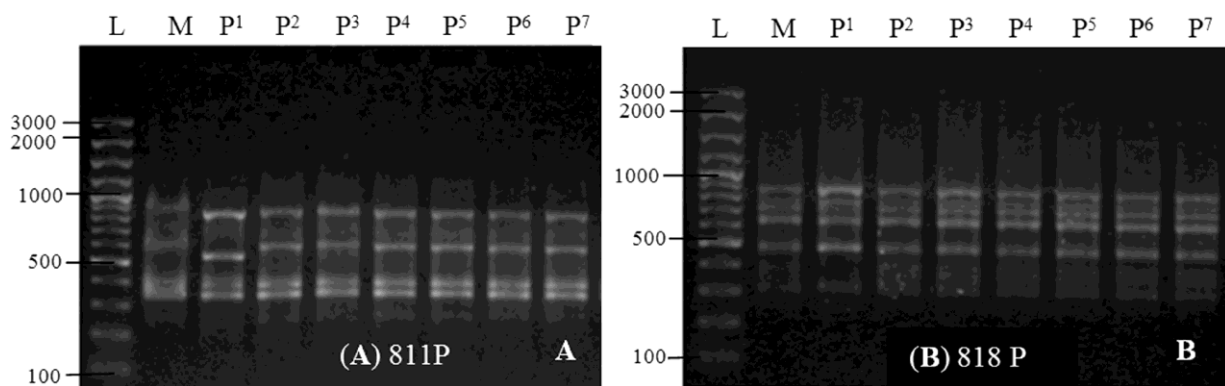


Fig. 1 — Fingerprinting profile of mother plant (M) and micropropagated plants via different pathways (P¹ Inter-nodal, P² Leaf, P³ Root, P⁴ Cotyledon, P⁵ Cotyledonary Node, P⁶ Hypocotyl, P⁷ nodal (Axillary bud) explant) of *Murraya koenigii*, generated by ISSR primers (A) 811P; and (B) 818 P. [L represents 100bp ladder]

Table 1 — Total number of amplified and polymorphic loci generated by selected 10 ISSR primers in micropropagated and mother plant of *Murraya koenigii*

Primer name	Sequence 5'-3'	Tm (°C)	Polymorphic Loci	Monomorphic Loci	Percentage polymorphism	Loci amplified	Approx. band range size (bp)
811P	(GA) ₈ C	52.8	00	06	00.00	48	250-850
814P	(CT) ₈ A	50.4	01	04	20.00	33	300-900
815P	(CT) ₈ G	52.8	02	07	22.22	60	350-1,750
818P	(CA) ₈ G	52.8	01	07	12.50	63	300-850
826P	(AC) ₈ C	52.8	00	05	00.00	40	100-1,500
UBC 810	(GA) ₈ T	50.4	00	04	00.00	32	250-1,000
UBC 842	(GA) ₈ YG	54.8	00	07	00.00	56	200-1,500
UBC 857	(AC) ₈ YG	54.8	02	02	50.00	24	400-1,000
UBC 880	(GGAGA) ₃	50.6	00	07	00.00	56	250-1,000
UBC 881	(GGGTG) ₃	58.8	01	06	14.29	53	500-2,000
			Total	07	55	465	

Table 2 — Jaccard's similarity coefficient of micropropagated and mother plants of *Murraya koenigii* by ISSR primers

	P ¹	P ²	P ³	P ⁴	P ⁵	P ⁶	P ⁷	M
P ¹	1.00							
P ²	0.91	1.00						
P ³	0.93	0.95	1.00					
P ⁴	0.91	1.00	0.95	1.00				
P ⁵	0.98	0.93	0.94	0.93	1.00			
P ⁶	0.98	0.93	0.94	0.93	1.00	1.00		
P ⁷	0.93	0.95	0.96	0.95	0.94	0.94	1.00	
M	0.96	0.91	0.93	0.91	0.98	0.98	0.96	1.00

[(M) Mother plant, P¹ - P⁷ micropropagated plants regenerated via different pathways (P¹ Inter-nodal, P² Leaf, P³ Root, P⁴ Cotyledon, P⁵ Cotyledonary Node, P⁶ Hypocotyl, P⁷ nodal (axillary bud) explant) of plant tissue culture]

Cluster analysis of ISSR data

The cluster constructed through NTSYS-pc presented in the form of dendrogram (Fig. 2) which illustrated the overall genetic fidelity or relationship among micropropagated and mother plants of *M. koenigii* patterns generated by 10 ISSR primers. The dendrogram was all the plants in two major cluster groups (group A and group B). Group A comprises of four plants M, P⁴, P⁵ and P⁷ which was further differentiated into two sub cluster, while group B included four plants (P¹, P² P³ and P⁶). Molecular markers have been used successfully to determine the degree of relatedness among individuals or group of accessions to clarify the genetic structure or variation among accessions, population, varieties and species^{24,33}.

ISSR markers can be used in population genetic studies of plant species as they effectively detect very low levels of genetic variation³⁴. On the basis of number, intensity and reproducibility of ISSR bands, 10 primers were selected out of the 25 primers tested in *M. koenigii*. The banding pattern of PCR amplified product from micropropagated plantlets was found to be monomorphic for most of the primer tested. Bands with same mobility were considered as identical irrespective of their band intensity.

Molecular markers have been utilized for detecting variation or confirmation of genetic fidelity during micropropagation³⁵. ISSR primer based assessment of regenerants has reported already in *Cornus alba*, *Cucumis melo* L., *Rauwolfia tetraphylla* L., *Simmondsia chinensis*, *Spilanthes calva*, *Tylophora indica* and *Zea mays*³⁶⁻⁴². The present report could be possibly the first report in which the comparative genetic fidelity analysis of regenerants are developed using all types of explants and mother plants of *M. koenigii* using ISSR markers.

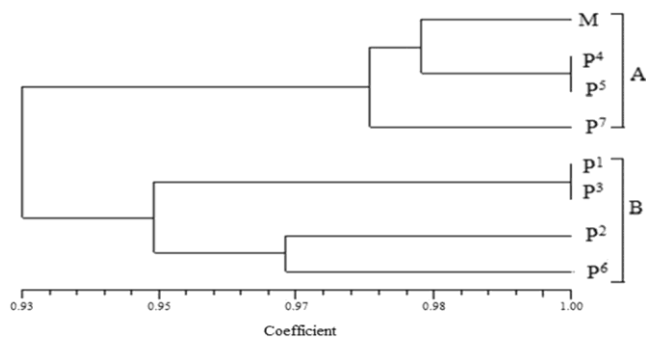


Fig. 2 — UPGMA dendrogram of micropropagated and mother plants of *Murraya koenigii* by ISSR primers

Genetic uniformity is one of the most important prerequisites for successful micropropagation of any plant species. Nevertheless, a major problem encountered in cells grown *in vitro* is the occurrence of genetic variation. Although the origin of variation is unclear, it points to two main factors; an intrinsic factor, while largely depends on the genetic fidelity of the explants and an intrinsic factor, depending on culture media and particularly PGRs⁴³.

The ISSR technique has been used successfully for analyzing the genetic fidelity of species propagated through *in vitro* shoot formation^{44,45}. Dendrogram was constructed on the basis of Jaccard's similarity matrix, followed by UPGMA based clustering analysis of ISSR profiles. The dendrogram showed the genotype of micropropagated and mother plant of *Murraya koenigii* were grouped into two major clusters A and B. Cluster A comprises with 3 groups and B with 3 groups. The ISSR data were also used to calculate genetic similarity between seven samples of micropropagated and mother plant of *Murraya koenigii* in pair-wise manner.

Conclusion

In this study, the true to the type nature of the *in vitro* raised micro-clones of *Murraya koenigii* was confirmed using ISSR markers. No variability was detected among the tissue culture-raised plantlets; hence, nodal explants can be successfully employed for the commercial multiplication of *Murraya koenigii* without much risk of genetic instability.

Acknowledgement

The authors are thankful to the University Grants Commission (UGC), New Delhi for financial support. The authors acknowledge Dr. Vinod Joshi, Scientist 'G',

Desert Medicine Research Centre, Jodhpur for extending necessary facilities to carry out this work. The authors are also thankful to Prof. SD Purohit, Head, Department of Botany M.L.S. University, Udaipur for statistical analysis of data.

Conflicts of interest

Authors declare that they have no conflict of interest.

References

- Chakrabarty M, Nath A, Khasnobis S, Chakrabarty M, Konda Y, Harigaya Y & Komiyama K, Carbazole alkaloids from *Murraya koenigii*. *Phytochemistry*, 46 (1997) 751.
- Nutan MTH, Hasnat A & Rashid MA, Antibacterial and cytotoxic activities of *Murraya koenigii*. *Fitoterapia*, 69 (1998) 173.
- Gupta GL & Nigam SS, Chemical examination of the leaves of *Murraya koenigii*. *Planta Med*, 19 (1970) 83.
- Rahman MM & Gray AI, A benzoisofuranone derivative and carbazole alkaloids from *Murraya koenigii* and their antimicrobial activity. *Phytochemistry*, 66 (2005) 1601.
- Tachibana Y, Kikuzaki H, Lajis NH & Nakatani N, Antioxidative activity of carbazoles from *Murraya koenigii* leaves. *J Agric Food Chem*, 49 (2003) 5589.
- Patel OP, Mishra A, Maurya R, Saini D, Pandey J, Taneja I, Raju KS, Kanoyiya S, Shukla SK, Srivastava MN & Wahajuddin M, Naturally occurring carbazole alkaloids from *Murraya koenigii* as potential antidiabetic agents. *J Nat Prod*, 79 (2016) 1276.
- Kyte L & Kleyn J, *Plants From Test Tubes: An Introduction to Micropropagation*. (Timber Press, Portland, Oregon, USA), 1996.
- Unal S, Turkmen G, Yagmur B, Bayraktar M & Gurel A, Improved *in vitro* propagation and direct acclimatization of *Cryptocoryne wendtii* in aquarium in the presence of aquarium fish *Puntius tetrazona* (Bleeker). *Indian J Exp Biol*, 57 (2019) 330.
- Khatik N & Joshi R, *In vitro* propagation of *Murraya koenigii* by axillary bud proliferation using mature explants. *Indian J Biotechnol*, 17 (2018) 379.
- Bhardwaj P, Jain CK & Mathur A, Comparative analysis of saponins, flavonoids, phenolics and antioxidant activities of field acclimatized and *in vitro* propagated *Bacopa monnieri* (L.) Pennell from different locations in India. *Indian J Exp Biol*, 57 (2019) 259.
- Kashyap S & Kale RD, Development of callus and cell suspension culture from the leaf of *Adhatoda vasica* Nees using economical growth media. *Indian J Exp Biol*, 57 (2019) 195.
- Jain P, Danwra K, Sharma HP & Mahato D, *In vitro* tissue culture studies and synthetic seed formation from *Plumbago zeylanica* L. *Indian J Exp Biol*, 56 (2018) 769.
- Larkin P & Scowcroft W, Somaclonal variation—a novel source of variability from cell cultures for plant improvement. *Theor Appl Genet*, 60 (1981) 197.
- Vasil IK, *Cell Culture and Somatic Cell Genetics of Plants*. Vol. 2, (Academic Press, Orlando), 1985, 330.
- Shenoy VB & Vasil IK, (1992). Biochemical and molecular analysis of plants derived from embryogenic cultures of napier grass (*Pennisetum purpureum* K. Sahum.). *Theor Appl Genet*, 83 (1992) 947.
- Bennici A, Anzidei M & Vendramin GG, Genetic stability and uniformity of *Foeniculum vulgare* Mill. regenerated plants through organogenesis and somatic embryogenesis. *Plant Sci*, 166 (2004) 221.
- Feyissa T, Welander M & Negash L, Genetic stability, *ex vitro* rooting and gene expression studies in *Hagenia abyssinica*. *Biol Plantarum*, 51 (2007) 15.
- Mathur P, Rao MS & Purohit SD, Genetic stability in microclones of *Celastrus paniculatus* Wild. derived from different explant sources as revealed by RAPD profiles. *Asian Australas. J Plant Sci Biotechnol*, 2 (2008) 91.
- Zucchi MI, Arizono H, Morais VA, Pelegrinelli FMH & Vieira MLC Genetic instability of sugarcane plants derived from meristem cultures. *Genet Mol Biol*, 25 (2002) 91.
- Anand A, Studies on genetic stability of micropropagated plants and reintroduction in an endemic and endangered taxon: *Syzygium travancoricum* Gamble (Myrtaceae). *J Plant Biotechnol*, 5 (2003) 201.
- Li DY, Kang DH, Yin QQ, Sun XW & Liang LQ, Microsatellite DNA marker analysis for genetic diversity in wild common carp (*Cyprinus carpio* L.) populations. *J Genet Genomics*, 34 (2007) 984.
- Sharma P & Sharma R, DNA fingerprinting of peach (*Prunus persica*) germplasm in accessing genetic variation using arbitrary oligonucleotide markers system. *Indian J Biotechnol*, 17 (2018) 484.
- Costa R, Pereira G, Garrido I, Tavares-de-Sousa MM & Espinosa F, Comparison of RAPD, ISSR, and AFLP Molecular Markers to Reveal and Classify Orchardgrass (*Dactylis glomerata* L.) Germplasm Variations. *PLoS One*, 11 (2016) e0152972.
- Rastogi RR, Singh N, Singh S, Pal AK, Roy RK & Rana TS, Assessment of genetic variability in the Bougainvillea varieties using morphological and molecular markers. *Indian J Exp Biol*, 57 (2019) 408.
- Nag S, Mitra J, Satya P, Kar CS & Karmakar PG, Genetic diversity assessment in jute (*Corchorus* species) utilizing inter simple sequence repeat and simple sequence repeat markers. *Indian J Biotechnol*, 17 (2018) 316.
- Chittora M, Assessment of genetic fidelity of long term micropropagated shoot cultures of *Achras sapota* L. var. 'Cricket Ball' as assessed by RAPD and ISSR markers. *Indian J Biotechnol*, 17 (2018) 492.
- Rostiana O, Niwa M & Marubashi W, Efficiency of Inter-Simple Sequence Repeat PCR for Detecting Somaclonal Variation among Leaf-Culture-Regenerated Plants of Horseradish. *Breed Sci*, 49 (1999) 245.
- Verma S & Rana TS, Genetic diversity within and among the wild populations of *Murraya koenigii* (L.) Spreng., as revealed by ISSR analysis. *Biochem Syst Ecol*, 39 (2011) 139.
- Ranade SA, Rana TS, Srivastava AP & Nair KN, Molecular differentiation in *Murraya koenigii* ex L., species in India inferred through ITS RAPD and DAMD analysis. *Curr Sci*, 90 (2006) 1253.
- Khatik N & Joshi R, An efficient protocol for *in vitro* shoot regeneration from different explants of *Murraya koenigii* (L.) Spreng. *J Phyto Res*, 30 (2017) 59.
- Saghai-Marouf MA, Soliman KM, Jorgensen RA & Allard RW, Ribosomal DNA spacer-length polymorphism in barley:

- Mendelian inheritance, chromosomal location and population dynamics. *Proc Natl Acad Sci*, 81 (1984) 014.
- 32 Duncan DB, Multiple range and multiple F tests. *Biometrics*, 11 (1955) 1.
- 33 Srivastava D, Gayatri MC & Sarangi SK, *In vitro* mutagenesis and characterization of mutants through morphological and genetic analysis in orchid *Aerides crispa* Lindl. *Indian J Exp Biol*, 56 (2018) 385.
- 34 Zietkiewicz E, Rafalski A & Labuda D, Genome fingerprinting by simple sequence repeat (SSR) -anchored polymerase chain reaction amplification. *Genomics*, 20 (1994) 176.
- 35 Tyagi RK, Agrawal AK, Mahalakshmi C, Hussain Z & Tyagi H, Low cost media for *in vitro* conservation of turmeric (*Curcuma longa* L.) and genetic stability assessment using RAPD markers. *In Vitro Cell Dev Biol Plant*, 43 (2007) 51.
- 36 Ilczuk A & Jacygrad E, *In vitro* propagation and assessment of genetic stability of acclimated plantlets of *Cornus alba* L. using RAPD and ISSR markers. *In Vitro Cell Dev Biol Plant*, 52 (2016) 379.
- 37 Raji MR, Lotfi M, Tohidfar M, Zahedi B, Carra A, Abbate L & Carimi F, Somatic embryogenesis of muskmelon (*Cucumis melo* L.) and genetic stability assessment of regenerants using flow cytometry and ISSR markers. *Protoplasma*, 255 (2018) 873.
- 38 Rohela GK, Jogam P, Bylla P & Reuben C, Indirect Regeneration and Assessment of Genetic Fidelity of Acclimated Plantlets by SCoT, ISSR, and RAPD Markers in *Rauwolfia tetraphylla* L.: An Endangered Medicinal Plant. *BioMed Res Int*, 2019 (2019) 1. <https://doi.org/10.1155/2019/3698742>.
- 39 Kumar S, Mangal M, Dhawan AK & Singh N, Assessment of genetic fidelity of micropropagated plants of *Simmondsia chinensis* (Link) Schneider using RAPD and ISSR markers. *Acta Physiol Plant*, 33 (2011) 2541.
- 40 Razaq M, Heikrujam M, Chetri SK & Agrawal V, *In vitro* clonal propagation and genetic fidelity of the regenerants of *Spilanthes calva* DC. using RAPD and ISSR marker. *Physiol Mol Biol Plants*, 19 (2013) 251.
- 41 Sharma MM, Verma RN, Singh A & Batra A, Assessment of clonal fidelity of *Tylophora indica* (Burm. f.) Merrill “*in vitro*” plantlets by ISSR molecular markers. *Springer Plus*, 3 (2014) 1.
- 42 Ramakrishnan M, Ceasar SA, Duraipandiyan V & Ignacimuthu S, Efficient plant regeneration from shoot apex explants of maize (*Zea mays*) and analysis of genetic fidelity of regenerated plants by ISSR markers. *Plant Cell Tissue Organ Cult*, 119 (2014) 183.
- 43 Smith MK, A review of factors influencing the genetic stability of micropropagated banana fruits. *Fruits*, 43 (1998) 219.
- 44 Podwyszynska M, Niedob K, Korbin M & Marasek A, Somaclonal variation in micropropagated tulips determined by phenotype and DNA markers. *Acta Horticulturae*, 714 (2016) 211.
- 45 Ahmad N, Wali SA & Anis M, *In vitro* production of true-to-type plants of *Vitex negundo* L. from nodal explants. *J Hort Sci Biotechnol*, 83 (2008) 313.